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Epidermal Cells of the Tail of an Anuran Larva Are Competent to Transform into the Adult-Type Cells

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ABSTRACT—The epidermis of an anuran tadpole shows the region-specific metamorphic fate: the body epidermis transforms into the adult epidermis, while the tail epidermis commits apoptosis. The only explanation for this difference has been that the tail lacks basal cells which are competent to transform into germinative cells of the adult epidermis. Human blood group antigen A was found to be a specific molecular marker for adult-type epidermal cells. A sharp threshold was apparent at the body-tail junction in expression of the antigen. Utilizing this marker, we succeeded in demonstrating the presence of a specific population of epidermal cells in the tail which can differentiate into the adult-type germinative cells under the direct action of thyroid hormone. This was an unexpected result in that the tail makes provision for the adult life. We propose a hypothesis on the regulation of region-specific metamorphic changes of the anuran larval epidermis.

INTRODUCTION

The metamorphic fate of the epidermis of an anuran tadpole depends on its location in the head-tail axis. Changes in the epidermis of the tail contrast with those in the epidermis of the body (head and body trunk region): the former is to be lost during metamorphosis, while the latter is to survive and transform into the adult type [10]. The present study was carried out to understand the reason for this different metamorphic fate of the two tissues at the cellular level.

The epidermis of a tadpole of bullfrog, *Rana catesbeiana,* is composed of three types of cells: apical, skein and basal cells [8, 10]. Apical and skein cells are larva-specific in that they terminate their life at the metamorphosis. Basal cells are the larva to adult cell in that they transform their characters from the larval type into the adult one. They are most likely the progenitor of germinative cells of the adult skin [8].

The only explanation given hitherto for the region-dependent metamorphic change in the epidermis described above has been the lack of basal cells in the tail and their presence in the body [8]. To verify if this explanation is correct, we looked for a specific probe that detects the adult-type epidermal cells, but not larval cells. We found that the human group A antigen is expressed specifically in the suprabasal cells of postmetamorphic skin and is qualified for the probe. Utilizing this probe, the present study could show the presence of a specific population of epidermal cells also in the tail that can differentiate into the adult-type germinative cells under the direct action of thyroid hormone. These cells are most likely basal cells. We suggest that the tail epidermis is competent to transform into the adult type.

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MATERIALS AND METHODS

The bullfrog, *Rana catesbeiana,* was used in the present study. Tadpoles were obtained from a local supplier and staged according to Taylor and Kollros [9].

Epidermal cells were prepared from the tail and the body of tadpole as described previously [6]. Cells were cultured in medium of RPMI-1640 (GIBCO) containing 7% fetal calf serum (Commonwealth Serum Laboratory) which had been treated with charcoal (Wako Pure Chemical Industries LTD.) to eliminate thyroid hormone as described previously [11]. RPMI medium was diluted with distilled water to 70% of its original strength. Cells were maintained at 24°C in a humidified incubator gassed with 5% CO_2 and 95% air. They were precultured for 2 days in the absence of 3,3′,5-triiodo-L-thyronine (T_3, Sigma Chemical Co.) and media were changed (day 0) with fresh medium with (experimentals) or without (controls) T_3. When the culture extended more than 4 days, media were changed at day 4.

Histological studies were performed as follows. The skin was removed from tadpoles, fixed with 10% neutralized formalin, embedded in paraffin and sectioned 4 μm thick. Staining with toluidine blue was performed according to Robinson and Heintzelman [8]. Sections for electron microscopy were prepared according to Kaiho *et al.* [2] and were viewed through a JE-100SX electron microscope. Sections for immunohistochemistry were also processed as described above. Cells for this aim were fixed with 10% neutralized formalin or methanol. The antigen A was detected by the method of Kaiho and Ishiyama [1] using anti-A antiserum (rabbit, Takeda) and Vecstastain ABC kits (Vector Lab.) as an indicator system.

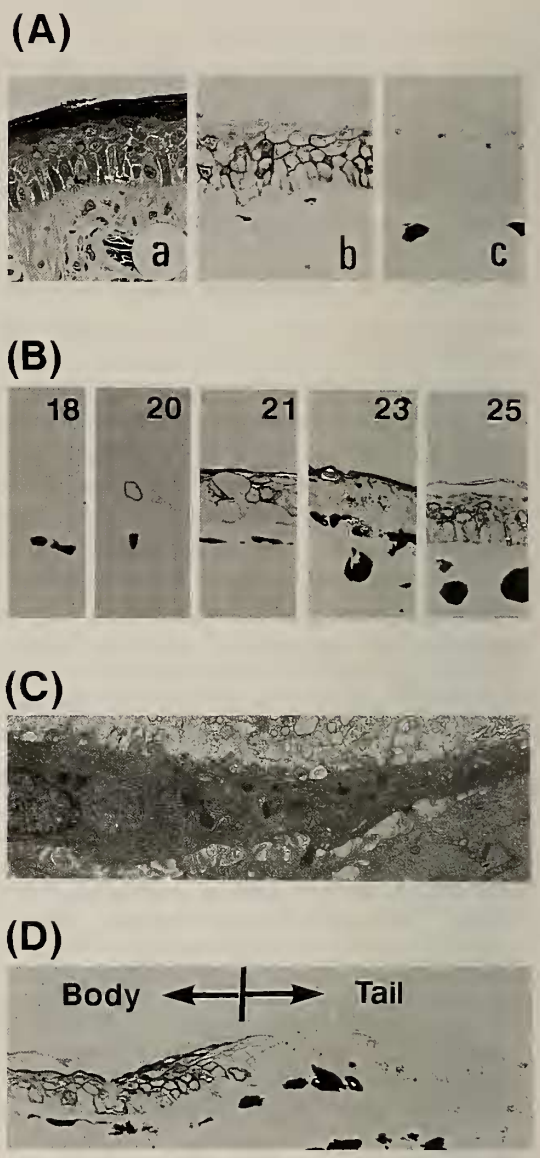

Fig. 1. Immunostaining of the epidermis by antisera against human blood group antigen A. (A) Cross section of the adult dorsal body skin. a, Section stained with toluidine blue, showing a typical five- or six- layered epidermal cells on the dermis. b, Immunostained section, showing that granular cells are positive but outermost keratinized and innermost germinative cells are negative. c, Negative control section stained with the anti-A antiserum which had been immunoabsorbed by human group A blood cells. Black granules in the epidermis were melanins of pigment cells. Magnification: 200×. (B) Immunostaining of the dorsal body skin of tadpoles during spontaneous metamorphosis. The number indicated in each figure shows the metamorphic stage defined by Taylor and Kollros [9]. A few positive cells appeared first at stage XX. The black-colored cells in the dermis are melanocytes. Magnification: 200×. (C) An electron micrograph of a cell which reacts with anti-A antiserum. Magnification: 2000×. (D) A section of the body-tail junction of the skin at stage XXI. The section was immunostained by anti-A antiserum. Black bodies under the epidermis are melanocytes. Note the clear immunological difference of the tail and the body epidermis. Magnification: 150×.

RESULTS AND DISCUSSION

It was found that the human blood group antigen A (A) is a useful probe for the analysis of transition of the epidermis during metamorphosis. Keratinizing cells in the stratum spinosum of the adult body skin of bullfrog were immunostained by the anti-A antiserum (Fig. 1A). This reaction was specifically immunoabsorbed by human A group blood cells. The keratinized outermost layer of the stratum corneum and the innermostlayer of the stratum basale were negative to the antiserum. These indicate that keratinizing daughter cells of basal cells in the germinative layer express human blood group antigen A.

Contrary to the adult skin, we found that there exist no epidermal cells positive to the antiserum in the entire skin of tadpoles at pre- and prometamorphic stages (Fig. 1B). A few positive cells appeared in the epidermis of the body at stage XX, which were flat in shape (Fig. 1C). The presence of A-positive cells at stage XX indicates the appearance of adult-type germinative cells in the body skin at this critical stage of metamorphosis, because they are differentiated daughter cells of germinative cells as shown in Figure 1A. The A-antigen expressing cells increased dramatically in their numbers at stage XXI through stage XXV, when the tail is lost completely, suggesting that basal cells are actively transforming into the germinative cells during climax stages of metamorphosis. It, therefore, can be concluded that the anti-A antiserum is a useful probe for the analysis of the conversion of basal cells from the larval type to the adult one during metamorphosis.

As expected, the tail epidermis did not contain the cells reactive to anti-A antibodies at stage XXI (the climax metamorphic stage) (Fig. 1D), indicating the absence of cells transforming into the adult type. As shown in the figure, the body and the tail epidermis was clearly separated at stage XXI for their reactivity to the antibody.

In vitro studies on epidermal cells clearly demonstrated that thyroid hormone (TH) is directly involved in the conversion of the larva-type epidermis to the adult type. The back skin was treated first with ethylenediaminetetraacetate (EDTA) to remove apical cells. Skein and basal cells were isolated from the EDTA-treated skin by digesting it further with trypsin and EDTA [6]. Cells thus obtained contained skein cells and basal cells with ratios of about 70% and 30%, respectively. They were cultured for 4 days in the presence of T_3 at the concentration of 10^{-8} M. The body cells were induced to express the antigens reactive to anti-A antiserum (Fig. 2, b and d). The identical *in vitro* study was performed for primary tail epidermal cells which were almost a homogeneous population of skein cells. Unexpectedly, tail epidermal cells were also induced to express the adult-type specific antigen as body cells (Fig. 2, a and c). Nishikawa *et al.* [5] recently obtained the similar result utilizing other adult-specific antigen as a prove. Epidermal keratin of *Xenopus laevis* with a molecular weight of 63 K is expressed in the adult but not in the larva. Tail epidermal cells of *X. laevis* were cultured in the presence of TH and shown to express 63 KDa

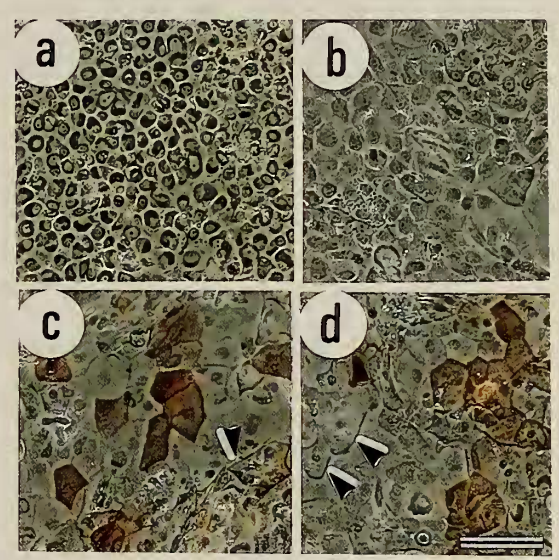

FIG. 2. Induction of anti-A positive cells in cultured epidermal cells. Eight hundred thousand epidermal cells obtained from tadpoles at stage X were cultured in 22-mm dishes at 24°C for 2 days. Media were changed and the culture was continued for additional 4 days in the presence or absence of 10^{-8} M T_3. Cells were immunostained with anti-A antiserum. a and c, tail cells; b and d, body cells; a and b, in the absence of T_3; c and d, in the presence of T_3. Anti-A positive cells are colored brown. Completely keratinized cells are not stained (arrowheads). Bars represent 100 μm.

keratin.

It is reasonable and expectable that the epidermis of the body contains the cells which express adult type characters under the influence of TH. Basal and skein cells in the primary culture seem to respond differently to TH. The former is stimulated to proliferate and then transform into the antigen-expressing adult-type cell, while the latter is fallen into the cell death by the direct action of TH [4]. The result of *in vitro* experiment for body epidermal cells coincides with that of *in vivo* experiment shown in Figure 1.

On the other hand, the *in vitro* result for the tail cells is against expectation in the following four reasons: the tail epidermis apparently need not provide for the adult-type cell, because its life ends up at metamorphosis [10]; the tail epidermis does not express the adult-type character *in vivo* at the climax stage of metamorphosis as shown above in Figure 1D; the tail epidermal primary culture does not contain basal cells as mentioned above; it was shown that the tail epidermis lacks basal cells [8]. However, the result shown in Figure 2 presents clear evidence that the tail contains a population of cells which is competent to convert to the adult type in response to TH.

The cells in the tail that expressed A-antigen in response to TH should be basal cells, because skein cells are to be subjected to the programmed cell death by the direct action of TH [4]. Two possibilities arise to explain the result of *in vitro* experiment of tail cells. One is that basal cells might be present in the tail cell population with so low frequencies as not to be detected micoscopically. This minor population of basal cells is stimulated by TH to proliferate and differentiate into the adult-type cells. The time course experiment on the A-antigen expression was performed. Few cells in the body but not in the tail expressed the antigen at day 0 (Fig. 3). These cells might be contaminated epidermal glandular cells which are known to exist in the body but not in the tail and produce A-antigens (Izutsu and Yoshizato, manuscript in submission). During 4 to 6 days in culture T_3 markedly enhanced the appearance of positive cells in both the tail and the body as in the case shown in Figure 2. It was noteworthy that a few positive cells appeared at day 4 to day 6 depending

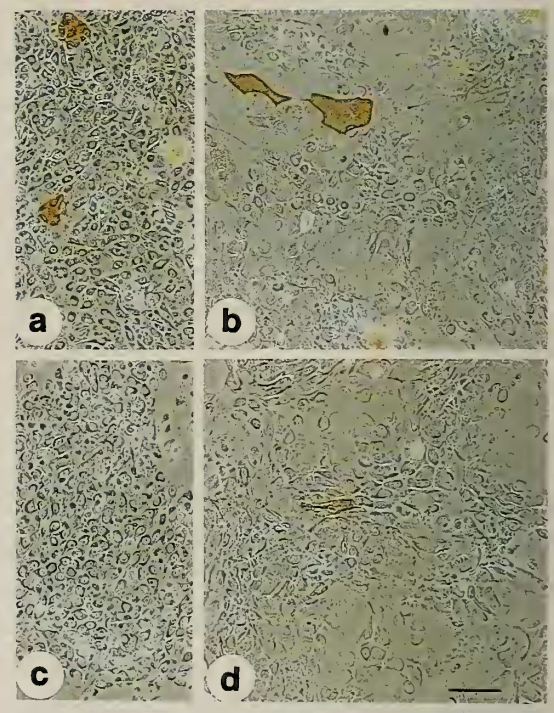

Fig. 3. Appearance of A-antigen expressing cells in tail and body epidermal cells in the absence of TH. Hundred thousand epidermal cells from tadpoles at stage X were cultured in 11-mm dishes for 2 days at 24°C. Media were changed (day 0) and the culture was continued for additional 6 days with a medium change at day 4. Cells were immunostained with anti-A antiserum. a and b, body cells; c and d, tail cells; a and c, day 0; b and d, day 6 without T_3. The bar equals 100 μm.

on experiments in a T_3-free culture of tail cells which contained about 10^5 cells (Fig. 3d). This supports the possibility described above that very few basal cells are included in the tail epidermis. Also a few additional positive cells appeared in T_3-free cultures of body cells, suggesting that the "differentiation" of the larval basal cell into the adult germinative cell could occur spontaneously at a very low level.

The second possibility is described below and is related to the difference between *in vivo* and *in vitro* status of epidermal cells. The reactivity of tail epidermal cells to anti-A antiserum was quite different between *in vivo* and *in vitro* experiments. As shown in Figure 1D, the tail epidermis *in vivo* does not show the positive reactivity even at the

climax stages of metamorphosis in which the plasma level of TH is high enough [7]. There might be some unknown factor(s) *in vivo* which suppresses the expression of A-antigen in the tail epidermis even in the presence of TH.

The character of the epidermis as tail or body seems to be determined by some mesenchymal factor. Utilizing transplantation techniques, Kinoshita *et al.* [3] revealed that the epidermis combined with the tail dermis undergoes degenerative changes during metamorphosis, irrespective of the origin of the epidermis (tail or body), while the tail epidermis combined with the body dermis transforms into the body-type and differentiates into the adult type. Based upon these we propose the second possibility that skein cells are not a homogeneous population: some skein cells could transform into basal cells. The epitheliomesenchymal interaction is postulated for this transformation. The body mesenchyme is required for the epidermal skein cell to "differentiate" into the basal cell. In contrast, the tail mesenchyme inhibits this transformation. When tail epidermal cells are removed from its mesenchyme and are cultured, some of them transform into basal cells because they are free from the influence of the mesenchyme. Thus, the tail epidermal cell in culture is competent to express A-antigens when exposed to TH.

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